THE CORRELATION OF THE AMOUNT OF SUNLIGHT WITH THE DIVISION RATES OF CILIATES.

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The analysis of the division rates of ciliates of three diverse orders which had been cultured under practically identical conditions, disclosed only a secular trend and a yearly cycle of change. The maximum of the yearly cycle [1] occurred during the month of July and the cycles of each organism were similar. The slopes of the secular trends were different for each organism. No evidence of the special "cycles" and "rhythms" which protozoölogists had reported in other investigations was found. This paper will show that the cycle of seasonal variation is closely related to the amount of sunshine recorded during the cycle and may perhaps be determined by solar radiation. Evidence from other investigations will be cited to support this conclusion.

I.

The division rates of *Paramecia aurelia* (mutant), *Blepharisma undulans,* and *Histrio complanatus* grown in pedigree isolation culture, with the same culture media, for three years, were obtained by Dawson.[2] These data were used in a previous analysis by Richards and Dawson.[3] The monthly averages of the sunlight recorded at the Boston and Block Island Stations of the U. S. Weather Bureau in terms of the per cent. of possible sunlight were furnished to me through the courtesy of Mr. G. A. Loveland. The figures for the Block Island Station for July and August were used as being the best available representation of the sunlight at Woods Hole, where Dawson kept the protozoa during

[1] *Rhythm* and *cycle* are here used with their usual meaning. The terms will be enclosed in quotation marks when given the special and restricted meaning found in the protozoölogical literature.

[2] Dawson, J. A., 1926, *J. Exp. Zoöl.,* XLIV., 133; 1926–27; XLVI., 345.

[3] Richards, O. W., and Dawson, J. A., 1927, *J. Gen. Physiol.,* X., 853.

the summer. The per cent. of possible sunlight, for each month, is the ratio of the recorded number of hours of sunlight to the maximum number of hours of sunlight that could occur that month if there was no cloudiness, etc. The per cent. of possible sunlight is used here because it saves the first step of the analysis, namely the converting of the number of hours into percentages.[4] This method includes and emphasizes the cycle of sunlight variation and introduces no significant error.[5]

The average yearly cycle of seasonal variation for each organism is shown in Fig. 1. The statistical methods used in obtain-

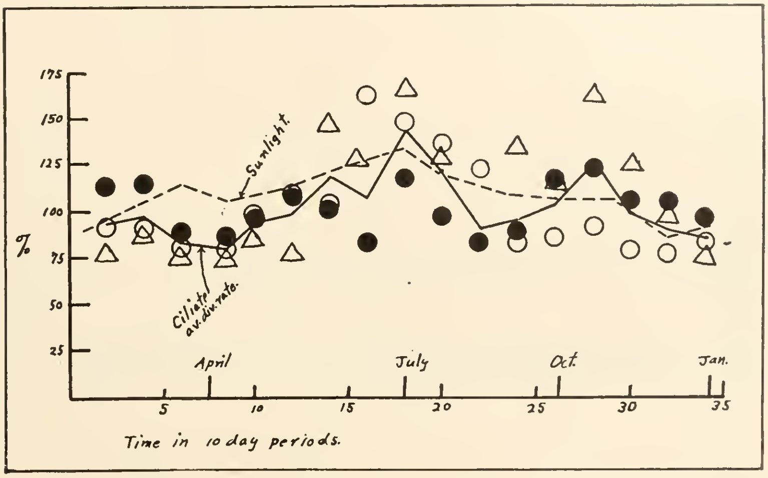

FIG. 1. The yearly cycle of seasonal variation for each organism and the average amount of sunlight. Note: maxima in July. o, *Paramecium*. ●, *Blepharisma*. Δ, *Histrio*.

ing this cycle, and employed for the further analysis of the sunlight data, are the same and are described in the previous paper.[3] The variation in the amount of sunlight is very similar to the variation in the magnitude of the average division rate of the

[4] *Cf.* previous analysis [3] and Rietz, H. L., Handbook of Mathematical Statistics, New York, 1924, 151 ff.

[5] When the means of the curve of percentage of possible sunlight and the curve of total hours of sunlight are superimposed the deviations of the monthly values from each other average 2.6 per cent. which is only 0.4 unit of Fig. 2*c* and does not effect the calculations. The mean absolute deviation is — 0.36 per cent. which shows that since the deviations almost cancel each other they may be ignored in this analysis.

organisms. Since the amount of sunlight for any given month was different in each year, as is shown in Fig. 2*a,* an accurate comparison can only be made by first removing the amount of the division rate cycle that corresponds to the amount of sunlight for each time and then comparing the residues. This is the procedure that was used in the original analysis of the data, except that this time we use the recorded amount of sunshine for each month instead of a generalized statistically determined cycle to eliminate the observed cyclic variation in the division rates which have been corrected for the secular trend, Fig. 2*b.* (This figure is identical with that of Fig. 2*d* of the original analysis.[3]) After the sunlight cycles, Fig. 2*a,* are removed from the division rate

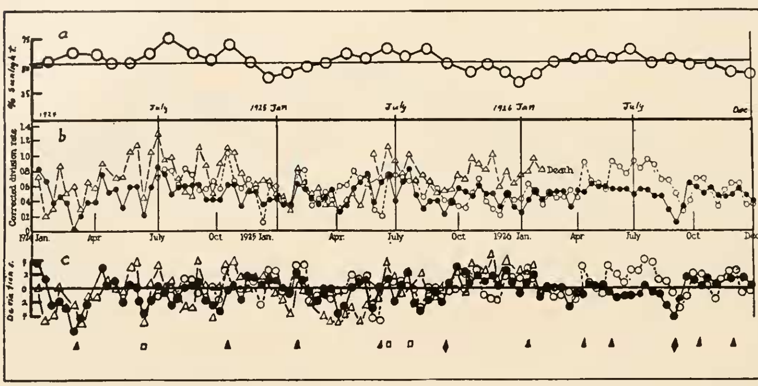

FIG. 2. *a,* the amount of sunlight. *b,* the division rate corrected for secular trend (*Cf.* Fig. 2*d,* Richards and Dawson [3]). *c,* division rate residues after minimiznig secular trend and variation associated with sunlight. o, *Paramecium.* ●, *Blepharisma.* △, *Histrio.* ▲, deviations associated with a change of water source. ■, deviations associated with removal to Woods Hole or Cambridge. ◆, deviations associated with temporary change of technician during the absence of Dawson.

data, Fig. 2*b,* there is left a residual amount of variation which is plotted as deviations from the superimposed mean division rate for each organism, in Fig. 2*c.*

II.

The residual curves may now be directly compared, as the disturbing effect of trend has been eliminated. If the amount of solar radiation determines the yearly cycle of seasonal variation in the division rates, all relation of the division rate curve of each organism to those of the others will have disappeared and the remaining deviations of the rates will be due to other influences not completely controlled by the culture technique.

The maximum correlation of the corrected *Paramecium* rates (x) with the *Blepharisma* rates (y) is their partial correlation independent of time, and is $r_{xy.t} = -.001$. The *Histrio* rates (z) may be correlated with the composite of the *Paramecium* and

TABLE I.

THE LEXIAN RATIOS * OF THE DIVISION RATES.

Organism.	Original Data.	Original Corrected Data.	Final Original Data.†	Corrected for Sunlight.	Final Corrected for Sunlight.†
Paramecium....	2.19	1.54	1.19	1.33	1.15
Blepharisma....	1.81	1.61	1.31	1.28	1.07
Histrio........	3.28	1.50	1.21	1.51	1.44

* The ratio of the relative standard deviation of the series to the relative Bernoulli standard deviation for the same series.

† These columns are for the corrected data less those deviations due to known disturbances (*Cf.* legend Fig. 2).

Blepharisma rates by means of the multiple correlation $r_{z.xy} = 0.08$. These coefficients demonstrate no relation between the corrected division rates. The unifying effect of the seasonal variation is removed. This removal is more complete than the removal of the statistically determined cycle of the previous analysis because the correlation coefficients of the original analysis [3] were -0.08 and 0.29, respectively. Consequently, the amount of sunlight seems to have ordered the similar yearly cycle of variation in the division rates of these representatives of three different orders of the ciliate infusoria.

III.

By means of the Lexian ratio the deviations of the residual curves of the previous analysis [3] were examined to disclose whether or not these remaining variations were due to chance experimentally uncontrolled factors alone or if they were due to definite unifying effect. The numerical values of the Lexian ratio are given in Table 1. When the deviations known to be caused by a change of medium, by removal from Cambridge to Woods Hole, or return, or by changes in culture technique are omitted the Lexian ratios more nearly approached unity. Purely chance deviations of a constant probability would give a Lexian ratio close to unity.

The Lexian ratios for the data of Fig. 2*c*, after the variations associated with the amount of sunlight are removed, are less than the figures of the previous analysis and, after the disturbances correlated with known causes are removed, the ratios are very nearly unity for all but the *Histrio* data. This probably indicates the inability of the *Histrio* to become adapted to the environment of the cultures, which ultimately resulted in its death.

The residual variation of the *Paramecium* and *Blepharisma* rates shows little disturbance beyond what might be attributed to chance influences. It is possible that if a corresponding ten day average, or, better, a running average of the sunlight for ten-day periods, were available and were used in place of the monthly averages of the sunlight, more of this remaining variation might have been removed. This analysis of the data leaves no trace of "cycles" or "rhythms" that might be attributed to cellular reorganization; and in fact none were observed by Dawson.[6] Consequently, it is an external influence, connected with sunlight, and not an internal organization that makes the inherently different division rates follow a uniform seasonal course. The division rate of the *Blepharisma* follows the amount of sunlight more closely than the others which may be due to the greater absorption of sunlight by the pigment of this organism.

IV.

This analysis shows that the yearly cycle of seasonal variation in the division rates of these ciliates, revealed in the original statistical analysis by Richards and Dawson,[3] is correlated with the variation in the amount of sunlight at different times of the year. The maximum of the division rate data occurs in July; likewise the maximum amount of sunlight in this region occurs in July (Fig. 1). This is not true in other localities. Wang[7] finds that

[6] Dawson, J. A., 1928, *J. Exp. Zoöl.*, LI., 199.

[7] Wang, C. C., 1928, *J. Morph. and Physiol.*, XLVI., 431.

there were more sunny days at Philadelphia during September and October, and further that there were more infusoria in the surface water of an open pond during these same months. His figures show that the number of infusoria is more closely related to the amount of sunshine than to the temperature of the water. I have superimposed his figures for temperature and for the number of infusoria, and have added the approximate amounts of

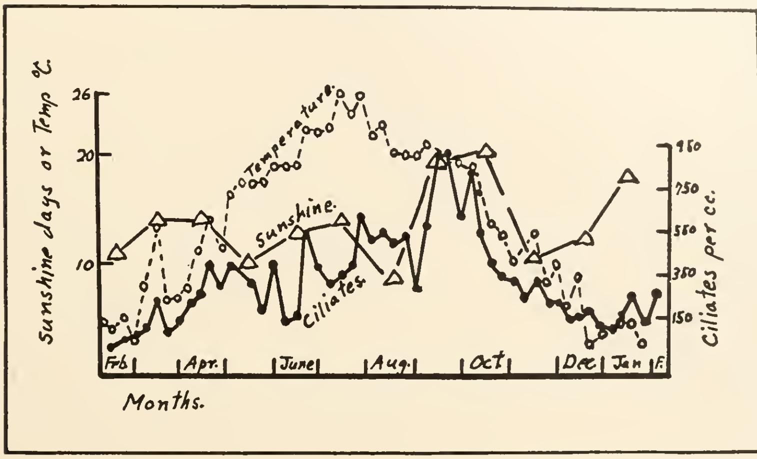

FIG. 3. Data from Wang.[7] The number of ciliates, temperature and amount of sunlight for the surface water of an open pond at Philadelphia. Note: Maximum number of organisms and sunlight in September and October. (*Cf.* Fig. 1).

sunshine in Fig. 3.[8] An inspection of the figure supports this conclusion.

Further corroborating evidence is to be found in another recent paper of Beers[9] who cultured *Didinium* under carefully controlled conditions and found no rhythms during a period of 265 days. The organisms were maintained in "diffused daylight" during the day time and in total darkness in an incubator at night. Dawson, however, kept his organisms in front of a window that received sunlight during a considerable part of the day protected

[8] Wang,[7] Chart 1, graph 1, and Chart 2, graph 3. He gives sunlight as days clear and partly cloudy. I have counted a partly cloudy day as about 55 per cent. of a clear day, which Dr. Brooks, meteorologist, Clark University, advises me may be less than the true value. The unsatisfactory histogram plot that Wang uses is avoided in Fig. 3. (*Cf.* Footnotes 3 and 6.)

[9] Beers, C. D., 1928, *J. Exp. Zoöl.*, LI., 485.

only by a drawn, light window curtain. Hence Dawson's data (used in the present study) would be expected to exhibit an effect of sunlight more clearly than would be expected with the more completely controlled environment used by Beers.

V.

No record of the temperature of Dawson's cultures is available. Since January and February, at Cambridge, are colder than the latter part of the year we might expect a lower rate of division early in the year, and this may account for the fact that the division rate is lower during the first quarter of the year than would be expected from the amount of sunshine at this time. This is also true of Wang's observations. The food supply of a pond is less in January and February than in the latter part of the year, which also explains part of this difference. Part of this deviation (Fig. 1) is due to the differences in amount of sunshine recorded for this part of the year for the different years. The drop in the composite division rate curve for June is due to the disturbance. of moving the cultures to Woods Hole. They are again disturbed when they are returned to Cambridge. The effect of the return is not as obvious because the time of return varied from year to year while the opening of classes brought Dawson to Woods Hole at essentially the same time each year. During September 1925 and 1926 the cultures were cared for by an assistant during the absence of Dawson with a resulting abnormal drop of the division rate during this month.

Examination of the division rates of these organisms since the original analysis shows that the secular trend has continued until almost the end of 1928 when a new upward trend begins.[4] The data are too few to establish this new trend. The yearly cycle of variation is less pronounced the longer the cultures are maintained which suggests an accumulative effect of some unfavorable influence, or, of some deficiency, in the protoplasm of these organisms. Such an effect would be more obvious with unicellular than with multicellular organisms owing to the direct continuity of the protoplasm of the former. This trend has persisted despite gradual improvement and refinement of the technique for culturing. The relation between the trend and the diminution of the magnitude of the cycle suggests that the loss of the ultra-violet or near ultra-violet rays may be the cause of the downward trend and diminished cycle of seasonal variation. The organisms are kept in glass moist chambers inside of a glass window so that a considerable part of the shorter wave lengths of light must be absorbed before reaching the animals. The influence of the shorter wave lengths of light on the division rate of protozoa could be determined by suitable experiment and should be evaluated in future studies made with these animals in more adequately controlled environments.

Summary.

1. Previous analysis of the division rates of *Paramecium aurelia* (mutant), *Blepharisma undulans,* and *Histrio complanatus* grown separately in pedigree isolation culture, under as nearly identical conditions as possible, for a period of 3 years, disclosed a secular trend and a seasonal rhythm for each organism. The seasonal rhythm has a maximum in July.

2. This seasonal rhythm is shown to be related to the amount of sunshine reaching the locality of the cultures. The maximum amount of sunshine is received in July also.

3. After the effect of trend and the influence of the amount of sunlight are removed from the division rates, they show no relation to each other except for deviations caused by known changes in the culture technique. Each organism has a division rate varying independently of the others, when the effect of external unifying influences are removed.

4. Consequently, the amount of sunlight, other conditions held constant, seems to determine the similarity of the division rate of these diverse organisms. The temperature is a secondary determining factor which has apparently less influence than sunlight when both variables are present in these experiments. Data from other investigations supports these conclusions.

5. It is suggested that the downward trend of the rates and the diminution of seasonal cycle which continue under laboratory conditions may be due to an accumulative deficiency of light of the shorter wave lengths which is absorbed by the containers, and that this effect be evaluated in studies made with more nearly constant environments.